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REVIEW ARTICLE

Effects of butyric acid on the periodontal tissue

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Summary Butyric acid, an extracellular metabolite from periodontopathic bacteria, induces apoptosis in murine thymocytes, splenic T-cells, as well as human Jurkat T-cells and peripheral blood mononuclear cells. Butyric acid-induced apoptosis is mediated by ceramide production, as well as reactive oxygen species (ROS) synthesis in mitochondria and subsequently JNK activation in MAP kinase cascades. Although the production of ROS and ceramide by themselves do not completely influence butyric acid-induced apoptosis, it can be concluded that ROS and ceramide production are the major contributors to butyric acid-induced apoptosis. Human gingival fibroblasts rescue butyric acid-induced T-cell apoptosis via proinflammatory cytokines, which are produced by fibroblasts stimulated with butyric acid. Moreover, T-cell adherence to fibroblasts is enhanced by butyric acids and butyric acid-induced T-cell apoptosis is down-regulated by T-cell adhesion to gingival fibroblasts. Butyric acid significantly suppresses the viability of inflamed gingival fibroblasts and induces apoptosis in a dose-dependent manner, whereas intact gingival fibroblasts isolated from healthy humans are resistant to butyric acid.

This review focuses on the effects of butyric acid and its possible contribution to destruction of gingival tissues and modulation of local immunity at gingival sites (175/max. 200).

Contents

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1. Introduction

Adult periodontitis is a chronic destructive disease involving host inflammatory responses to Gram-negative bacteria. Recent studies in periodontal medicine suggest an association between human periodontal disease and certain systemic disorders such as diabetes mellitus, pneumonia, heart disease and preterm birth [1]. The severe destructive adult

Table 1 Concentration of short-chain fatty acids in the culture supernatant of each bacterial strain.

Culture supernatants	Short-chain fatty acids level (mM)[a]					
	Acetic	Propionic	*n*-Butyric	*iso*-Butyric	*n*-Valeric	*iso*-Valeric
BHI[b]	4.9 ± 0.2	ND[c]	0.5 ± 0.1	ND	ND	ND
P. gingivalis FDC381 (24 h)[d]	12.6 ± 0.4	2.2 ± 0.2	18.1 ± 0.6	4.7 ± 0.3	ND	9.8 ± 0.5
P. gingivalis FDC381 (48 h)	12.7 ± 0.3	5.9 ± 0.2	27.1 ± 0.9	7.5 ± 0.3	ND	14.7 ± 0.3
P. gingivalis W83	13.2 ± 1.3	5.8 ± 0.1	21.0 ± 1.8	3.8 ± 0.4	0.3 ± 0.1	12.6 ± 0.2
P. gingivalis ATCC 33277	8.5 ± 0.2	2.5 ± 0.1	18.9 ± 1.6	2.8 ± 0.2	ND	6.7 ± 0.1
P. intermedia ATCC 25611	12.8 ± 2.4	19.1 ± 2.0	0.1 ± 0.1	4.7 ± 0.4	ND	3.4 ± 0.3
P. nigrescens ATCC 33563	15.4 ± 1.0	ND	ND	1.5 ± 0.2	ND	4.5 ± 0.1
F. nucleatum ATCC 33568	16.1 ± 1.5	4.6 ± 0.3	26.8 ± 1.9	ND	0.2 ± 0.1	ND
F. nucleatum ATCC 23726	12.2 ± 1.0	3.3 ± 0.2	21.3 ± 2.8	ND	ND	ND
C. ochracea ATCC 33596	8.6 ± 1.2	0.7 ± 0.3	ND	ND	ND	0.2 ± 0.1
A. a.[e] ATCC 43718 (Y4)	14.1 ± 1.3	ND	ND	ND	ND	ND
E. coli K235	5.2 ± 0.3	ND	ND	ND	ND	ND

[a] SCFAs present in the 48-h cultured supernatants were analyzed by gas-chromatography. Each measurement was based on the results of three independent experiments; results are mean ± SD.
[b] Brain Heart Infusion used as control.
[c] Not detected.
[d] *P. gingivalis* FDC 381 cultures grown for either 24 or 48 h.
[e] *Aggregatibacter actinomycetemcomitans*.

Table 2 Cellular differences in sensitivity to apoptosis induced by culture supernatants.

Culture supernatants	DNA fragmentation (%)[a]				
	Jurkat	U-937	THP-1	Ca9-22	Gin 1[b]
BHI[c]	7.2 ± 1.1	5.4 ± 0.4	6.4 ± 0.4	8.9 ± 0.2	8.2 ± 4.1
P. gingivalis FDC381	28.4 ± 2.3	24.2 ± 2.1	24.4 ± 2.1	NT[d]	NT
P. gingivalis W83	23.4 ± 2.8	27.5 ± 2.8	27.5 ± 1.8	NT	NT
P. gingivalis ATCC 33277	28.9 ± 1.7	21.4 ± 3.2	28.8 ± 2.0	9.8 ± 1.8	9.5 ± 3.8
P. intermedia ATCC 25611	7.4 ± 1.2	6.9 ± 0.8	8.8 ± 0.4	8.4 ± 1.8	7.4 ± 4.4
P. nigrescens ATCC 33563	6.8 ± 1.3	4.9 ± 0.7	6.8 ± 0.8	8.2 ± 2.1	8.2 ± 5.1
F. nucleatum ATCC 33568	58.4 ± 3.2	36.4 ± 1.8	37.2 ± 1.5	NT	NT
F. nucleatum ATCC 23726	55.8 ± 2.8	38.2 ± 2.1	39.8 ± 3.1	11.1 ± 1.4	11.1 ± 3.4
C. ochracea ATCC 33596	7.4 ± 1.8	6.8 ± 0.8	6.3 ± 0.4	NT	NT
A. a.[e] ATCC 43718 (Y4)	9.8 ± 1.4	7.2 ± 0.5	7.1 ± 0.9	9.2 ± 4.3	11.5 ± 4.1
E. coli K235	7.6 ± 0.8	5.5 ± 0.4	8.2 ± 1.2	NT	NT
Butyric acid[f] 1.25 mM	12.4 ± 2.6	9.8 ± 1.2	19.8 ± 2.8	9.1 ± 3.2	6.2 ± 2.8
5.0 mM	28.9 ± 3.1	26.5 ± 2.4	28.4 ± 3.4	10.8 ± 2.8	11.2 ± 3.1

[a] Cells were treated with 48-h cultured supernatants for 21 h. Harvested cells were assayed by the DPA assay (46). Each measurement was based on the results of three independent experiments; results are mean ± SD.
[b] Human cell lines: Jurkat; T lymphoma cell, U937 and THP-1; monocytic leukemia cell, Ca9-22; oral epitherial carcinoma cell, Gin 1; gingival fibroblast.
[c] Brain Heart Infusion used as control.
[d] Not tested.
[e] *Aggregatibacter actinomycetemcomitans*.
[f] Sodium butyrate.

periodontitis is caused by a mixed bacterial infection with a combination of certain periodontopathogens [1]. These bacteria produce a variety of virulence factors such as proteases, lipopolysaccharides (LPS), fimbriae, and butyric acid.

Butyric acid is one of the short-chain fatty acid (SCFA), an extracellular metabolite from anaerobic bacteria, and described as a saturated unbranched alkyl monocarboxylic acid of four carbon atoms [2]. Most of the work on SCFA has been performed with the intestinal microflora of human and animal hindguts. Butyric acid exhibits several unexpected properties when added to cells in culture and also *in vivo*. The main effects can be summarized as follows: (1) arrest of cell-proliferation and induction of apoptosis, (2) alteration of cell morphology and ultrastructure, and (3) alteration of gene expression. It is important to note that all of the effects of butyric acid are reversible. The other SCFA are much less effective or not effective at all in cell growth and gene expression. Butyric acid strongly inhibits histone deacetylases (HDACs), leading to the alteration in chromosomal stricture and gene expression [3,4].

Periodontopathic bacteria produce high levels of butyric acid and induces apoptosis in murine- and human T- and B-cells [5–7]. Emerging evidence indicates the bacterial modulation of apoptosis in an important part of pathogenesis. Specific pathogens or their extracellular products may induce host cell apoptosis [8,9]. For example, apoptosis has been observed in T-cells from patients with AIDS, as well as in activated peripheral blood lymphocyte cultures infected with HIV-1 [10,11]. In chronic inflammatory disorders, individuals with chronic hepatitis C have high hepatocyte loss due to apoptosis [12]. Bacteria such as *Helicobacter pylori* can also induce macrophage apoptosis [13]. Apoptosis is a key event in the regulation of the lifespan of terminally differentiated leukocytes in chronic inflamed human gingival tissue [14,15].

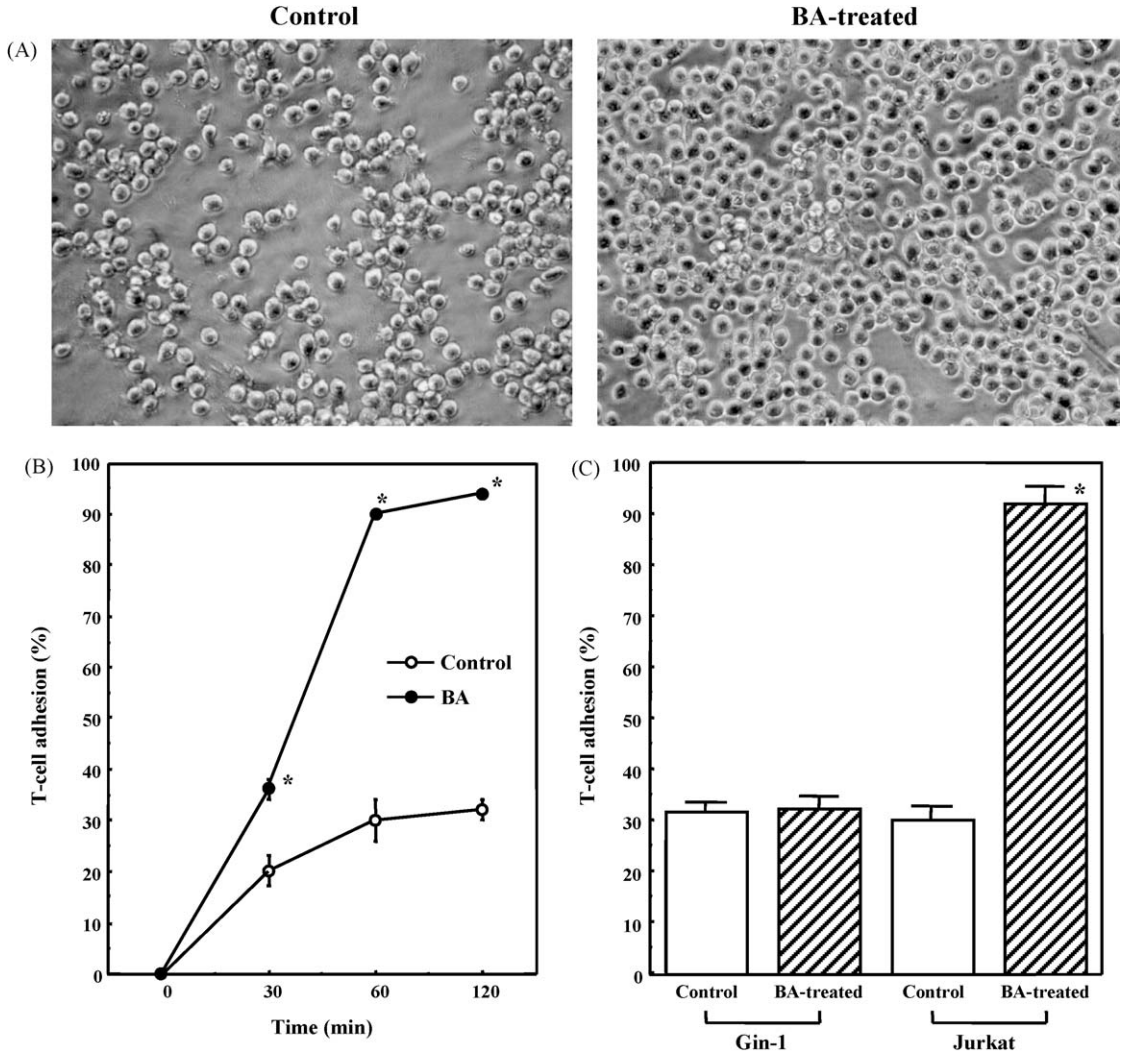

Figure 1 Effect of butyric acid on Jurkat cell adhesion to human fibroblast Gin 1 cell monolayer. Jurkat cells were directly co-cultured with Gin 1 cells in the presence or absence of 5 mM butyric acid for 30, 60 and 120 min. The number of Jurkat cells adherent to Gin 1 cells was counted under a phase-contrast microscope (A and B). In other experiments, Jurkat or Gin 1 cells were pretreated with 5 mM butyric acid for 2 h, and then co-cultured with Gin 1 or Jurkat cells, respectively, for 1 h. The number of Jurkat cells adherent to Gin 1 cells was counted by using a phase-contrast microscope. The results are expressed as the means ± standard errors of the means (erro bars) of three different experiments with triplicate cultures. Values that were significantly different from those of corresponding negative controls at $P < 0.01$ are indicated by asterisks.

This review focuses on the possible contribution of butyric acid to the destruction of gingival tissues and the modulation of local immunity at gingival sites.

2. Metabolic by-products of periodontopathic bacteria

Previous investigations showed that the butyric acid concentration in subgingival plaque from a periodontitis site can reach 14.4—20 mM, and that its concentration in periodontal pockets is correlated with the severity of periodontal diseases [16—18]. Butyric acid was proposed to be an important virulence factor in these periodontopathogens.

The respective metabolism of periodontopathic bacteria such as *Porphyromonas*, *Prevotella*, and *Fusobacterium* spp., is characterized by the production of an identifiable fingerprint of SCFA, which are major by-products of anaerobic metabolism that are released into the microenvironment at the infection site [19—21]. Previous studies have demonstrated that these SCFA exert inhibitory effects on gingival fibroblast proliferation and phagocytosis. These studies also demonstrated that SCFA present in the culture filtrates of *Porphyromonas gingivalis*, *Prevotella losheii*, and *Fusobacterium nucleatum*, greatly inhibited murine T- and B-cell-proliferation and cytokine production [22] (Table 1).

3. Butyric acid-induced apoptosis

3.1. Cellular differences in sensitivity to apoptosis induced by butyric acid

The addition of high concentrations of butyric acid (5 mM) inhibited murine T- and B-cell-proliferation and -induced apoptosis [5,6]. Similar results were obtained with the cell lines of macrophages and monocytes [14]. However, fibroblasts and oral epithelial cells are resistant to butyric acid-induced apoptosis. Nevertheless, inflamed gingival fibroblasts from individuals with adult periodontitis were highly susceptible to mitochondria- and caspase-dependent apoptosis induced by butyric acid, compared to healthy gingival fibroblasts [23] (Table 2).

3.2. Cellular events involved in butyric acid-induced apoptosis

We previously investigated the contribution of reactive oxygen species (ROS), mitochondria, ceramide and MAP kinases in butyric acid-induced human Jurkat cell apoptosis [7]. Following exposure of the cells to butyric acid, a pronounced accumulation of ROS was observed. Exposure of cells to butyric acid resulted in an increase in cellular ceramide concentrations in a time-dependent fashion. Butyric acid-induced apoptosis was inhibited by a potent inhibitor of sphingosine kinase. Using anti-extracellular signal-regulated kinase (ERK), anti-c-Jun N-terminal kinase (JNK), and anti-p38 phosphospecific antibodies, we showed a decrease in ERK, but not in JNK and p38 phosphorylation after treatment of cells with butyric acid. Pretreatment of cells with the JNK inhibitor attenuated the effect of butyric acid on apoptosis, whereas no effect was seen with the p38 inhibitor or the ERK inhibitor. These data indicate that butyric acid-induced T-cell apoptosis is mediated by ceramide production, ROS synthesis in mitochondria, and JNK activation in the MAP kinase cascade. These results were further confirmed by the expression profile of butyric acid-treated Jurkat cells obtained by means of cDNA arrays [24—29].

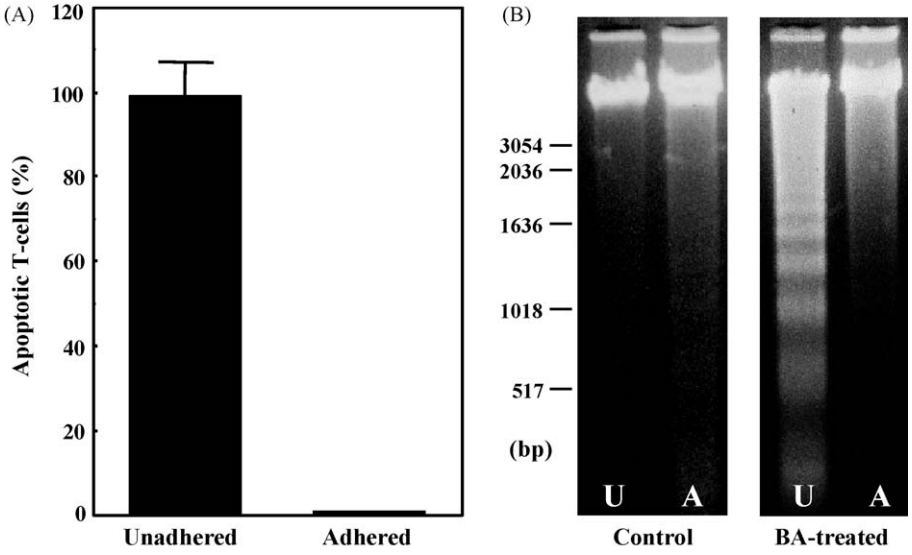

Figure 2 Analysis of Jurkat cells that adhere to human gingival fibroblast Gin 1 cells. Jurkat cells were directly co-cultured with Gin 1 cells in the presence of 5 mM butyric acid. The viability of Jurkat cells which adhered or unadhered to Gin 1 cells was examined by SYTOX green nucleic acid staining (A), followed by confocal laser scanning microscopy (31). The results are expressed as the means ± standard errors of the means (error bars) of three different experiments with triplicate cultures. The viability of Jurkat cells that were adherent or non-adherent to Gin 1 cells was also examined by agarose gel electrophoresis of DNA extracted from Jurkat cells (B). Lane U, unadhered T-cells; lane A, adhered T-cells.

4. Importance of cell-cell communication in inhibiting T-cell apoptosis

Previous reports have demonstrated the direct and indirect effects of human gingival fibroblasts (HGF) on the susceptibility of T-cells to butyric acid-induced apoptosis. We have reported that human gingival fibroblasts rescue butyric acid-induced T-cell apoptosis via proinflammatory cytokines such as interleukin (IL) -6 and IL-11, which are produced by fibroblasts stimulated with butyric acid [30]. The number of Jurkat T-cells adherent to HGFs was significantly increased following the addition of butyric acid. All Jurkat cells that adhered to HGFs remained viable, while the non-adherent Jurkat cells exhibited marked apoptosis. The increase in T-cell adhesion to HGFs was also observed when Jurkat cells, but not Gin 1 cells, were pretreated with butyric acid. The expression levels of CD44, very late antigen (VLA)-2 and VLA-5, but not leukocyte function-associated antigen 1 (LFA-1) and VLA-4 expression on Jurkat cells were increased following treatment with butyric acid [31—37]. These results indicate that T-cell adherence to fibroblasts is enhanced by butyric acid, and that butyric acid-induced T-cell apoptosis is down-regulated by T-cell adhesion to HGFs through an interaction with the adhesion molecules CD44, VLA-2 and VLA-5 expressed on T-cells stimulated with butyric acid (Figs. 1—3).

5. Effects of butyric acid on microbial interactions

SCFAs inhibit the growth of several strains of oral streptococci and modulate biofilm-formation (unpublished data). Recent studies showed that latently infected cells harbor the HIV-1 proviral DNA genome primarily integrated into heterochromatin, allowing the persistence of transcriptionally silent proviruses [38,39]. It was demonstrated that HDAC inhibitors such as trichostatin A and sodium butyrate could induce viral gene expression [40—43]. Hypoacetylation of HDACs is

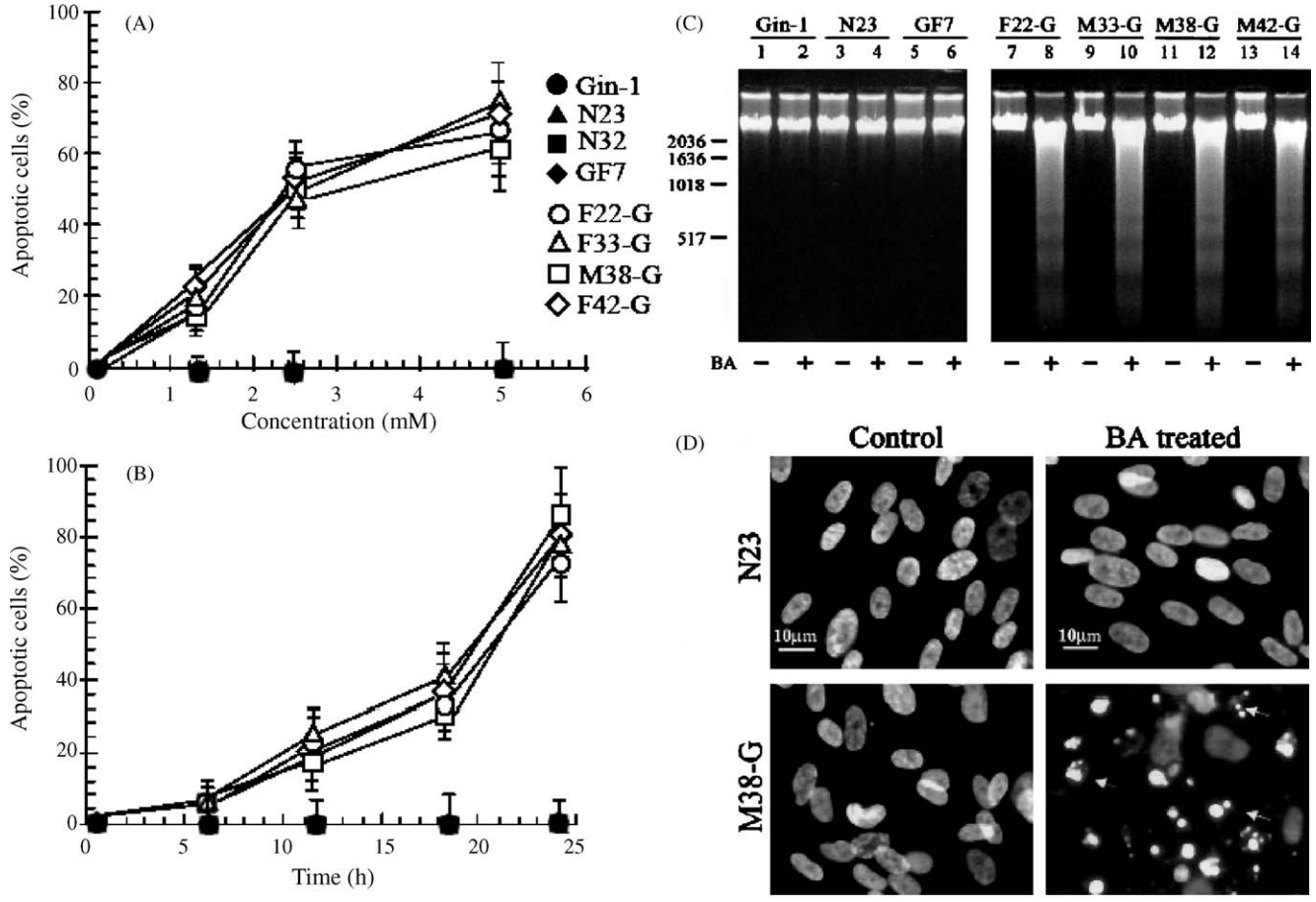

Figure 3 Butyric acid induces apoptosis in inflamed gingival fibroblasts. (A) Healthy gingival fibroblasts (HGF; Gin-1, N23, N32, and GF7) and inflamed gingival fibroblasts (IGF; F22-G, F33-G, M38-G, and F42-G) were cultured (A) with the indicated amount of butyric acid for 24 h and (B) in the presence of 5 mM butyric acid for the indicated time periods. Harvested cells were stained with DAPI and counted. The results are expressed as the mean ± errors of the means (error bars) of three different experiments. (C) Agarose gel electrophoresis of DNA. HGFs (Gin-1, N23, and GF7) and IGFs (F22-G, F33-G, M38-G, and F42-G) were cultured in the absence (lanes 1, 3, 5, 7, 9, 11, and 13) or presence (lanes 2, 4, 6, 8, 10, 12, and 14) of 5 mM butyric acid (BA). (D) Nuclear morphologies of gingival fibroblasts treated with butyric acid. HGF (N23, upper panels) and IGF (M38-G, lower panels) were cultured in the absence (left panels) or presence (right panels) of 5 mM butyric acid (BA) and stained with DAPI. Representative results from the three independent experiments. The scale bars, 10 μm.

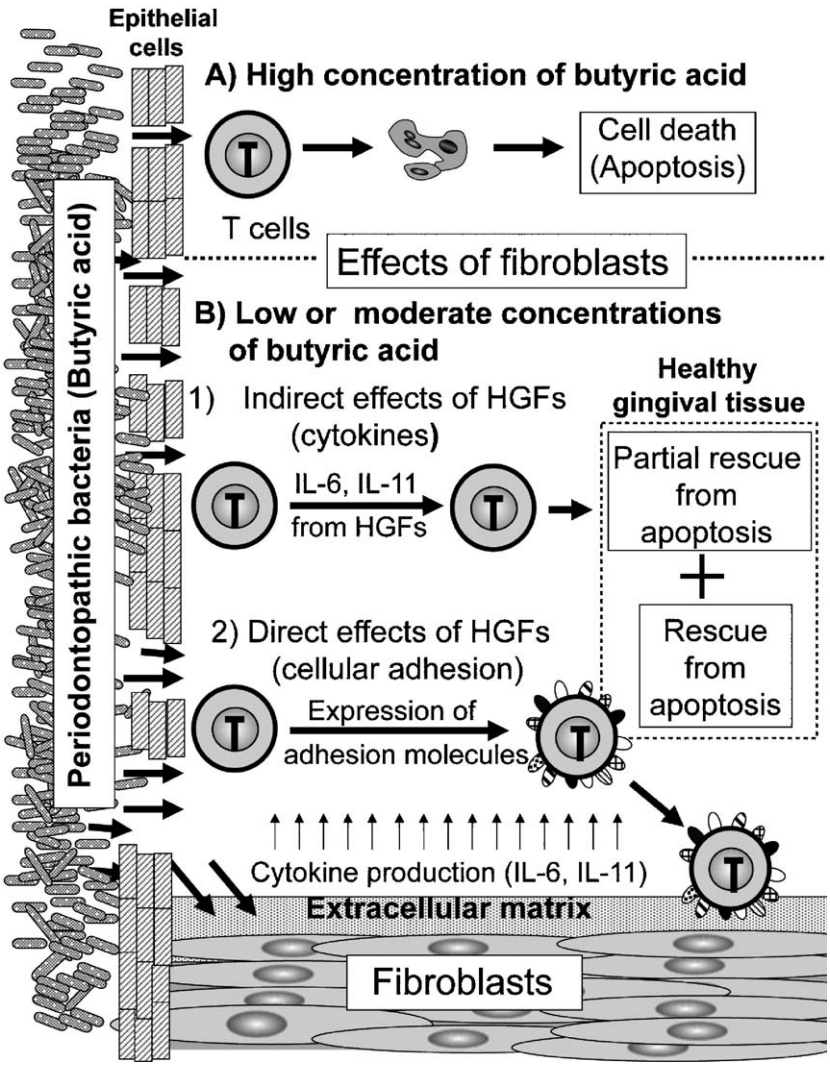

Figure 4 Effects of butyric acid on the periodontal tissue.

involved in the maintenance of HIV-1 latency by repressing viral transcription [40,41,44]. Chromatin immunoprecipitation assays revealed that the corepressor complex containing HDAC1 and AP-4 was dissociated from the HIV-1 long terminal repeat promoter upon stimulation with culture supernatant of *P. gingivalis* containing butyric acid [45]. *P. gingivalis*-infection could induce HIV-1 reactivation via chromatin modification and butyric acid appears to be responsible for this effect. Therefore, periodontal diseases could be a risk factor for HIV-1 reactivation in infected individuals and might contribute to the systemic dissemination of the virus.

6. Conclusions

Butyric acid, a metabolic by-product of periodontopathic bacteria, causes many different effects on periodontal tissue. In the series of our experiments, we clearly demonstrated that high concentration of butyric acid induces cytotoxicity and apoptosis in many different cell types. Some of the properties of butyric acid, *i.e.*, its ability to alter chromosomal structure and gene expression in many different cell types, may influence the microenvironment at the infection sites of periodontopathic bacteria. Therefore, butyric acid could contribute to the destruction of gingival tissues, induce alterations in immunomodulation, and play a role in certain systemic diseases as well as in microbial infections. In the contrast to these effects, low or modulate concentrations of butyric acid induce profitable effects on periodontal tissue such as promotion of cell growth and healing of injured tissue (data not shown). Moreover, butyric acid-induced T-cell apoptosis is down-regulated by T-cell adhesion to gingival fibroblasts through an interaction with the adhesion molecules, and proinflammatory cytokines that produced by fibroblasts in the healthy gingival tissue (Fig. 4).

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